European Journal of Clinical Nutrition (2012) **66,** 862–868

www.nature.com/ejcn

ORIGINAL ARTICLE

Effects of 25OHD concentrations on chances of pregnancy and pregnancy outcomes: a cohort study in healthy Danish women

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BACKGROUND/OBJECTIVES: Plasma 25-hydroxyvitamin D (P-25OHD) concentrations may affect pregnancy outcomes. To elucidate this further, we studied the effects of pre-conception P-25OHD concentrations on chances for pregnancy as well as the effects of P-25OHD during pregnancy on the risk of miscarriage, birth weight and length, Apgar score and head circumference. Moreover, we studied whether pregnancy and breastfeeding patterns affect maternal P-25OHD concentrations.
SUBJECTS/METHODS: A total of 153 healthy Caucasian women with pregnancy plans were followed with measurements performed before pregnancy, at pregnancy weeks 11 ± 2, 22 ± 1 and 35 ± 2 as well as 15 ± 7, 129 ± 12 and 280 ± 15 days postpartum. Furthermore, 75 non-pregnant, age-matched women were followed in parallel as controls.
RESULTS: The 203 women were aged 29 (25–35) years. At baseline, median P-25OHD was 59 nmol/l. Of these women, 31% had P-25OHD <50 nmol/l, whereas 12% had levels above 80 nmol/l. Within $\sim$6 months after inclusion, 63% conceived. P-25OHD was not associated with chances of conceiving or overall risk of miscarriage. However, women with a miscarriage in their second trimester ($n=3$) had lower P-25OHD concentrations at measurements performed in the first trimester compared with women without a miscarriage ($P=0.03$). P-25OHD before or during pregnancy was not associated with gestational length or infant parameters. Adjustments for possible confounders did not change the result. During pregnancy, P-25OHD changed significant over time, but similar changes occurred within the control group, indicating no effect of pregnancy *per se* ($P=0.59$). Overall, P-25OHD did not differ according to length of breastfeeding at 2 weeks, and 4 and 9 months postpartum, although women breastfeeding for >9 months had lower P-25OHD levels at the last visit compared with the controls.
CONCLUSION: P-25OHD concentrations did not affect fertility or pregnancy outcomes, although low P-25OHD may be associated with an increased risk of late miscarriage.

European Journal of Clinical Nutrition (2012) **66,** 862–868; doi:10.1038/ejcn.2012.18; published online 29 February 2012

Keywords: vitamin D; 25-hydroxyvitamin D; chances of pregnancy; birth weight and length; Apgar score; lactation

INTRODUCTION

Vitamin D is of importance for multiple health outcomes. Only few data are available on the possible effects of plasma 25-hydroxyvitamin D (P-25OHD) on fertility.[1] In female rats, low P-25OHD concentration has been associated with gonadal insufficiency,[2] although it is unclear whether this is due to the effects of hypocalcemia rather than a direct effect of P-25OHD. The vitamin D receptor is expressed in human sperm cells, and 1,25-dihydroxyvitamin D concentration may affect sperm survival and the acquisition of fertilizing ability of sperm cells.[3,4]

Vitamin D status is assessed by measuring P-25OHD concentration[5] and is considered to be replete at P-25OHD concentrations >50 nmol/l, although recent studies have suggested that only P-25OHD >80 nmol/l should be considered as an optimal vitamin D status for non-skeletal outcomes.[6] P-25OHD insufficiency is widespread in all age groups, including younger women with childbearing potential. Several studies, including a prior Danish study, have shown that one out of three women who recently have given birth have P-25OHD concentrations <50 nmol/l.[7–9]

Low P-25OHD concentration in uterus has been linked to an increased risk of type I diabetes,[10] asthma[11,12] and other chronic diseases later in life,[13] as well as immediate adverse outcomes of pregnancy, including an increased risk of miscarriage, pre-eclampsia, gestational diabetes mellitus and preterm birth.[14,15] In several,[7,16–19] although not all,[20–23] studies, a positive association has been shown between vitamin D intake or P-25OHD concentration and head circumference, weight and length of the newborn infant. The positive effects of improved P-25OHD concentration on pregnancy outcome were also shown in a British double-blind randomized trial.[24] The study included pregnant Asian women with P-25OHD deficiency, randomized to oral ergocalciferol (D_2), 1000 IU/day or placebo during the last trimester[24] of their pregnancy. Vitamin D supplements improved maternal weight gain and lowered the risk of giving birth to an infant small for gestational age. Similarly, in a cross-sectional study, including 449 pregnant women, maternal calcium and vitamin D intake was associated with weight gain of mothers during pregnancy, as well as birth weight and 1-min Apgar score.[25]

Until now, no firm data have answered the question of whether pregnancy and lactation lead to an increased need for vitamin D.[26–28]

In order to study possible effects of P-25OHD concentration on human fertility and pregnancy outcome, we recruited healthy young Danish women, with immediate plans for pregnancy. Within this cohort, we studied the effects of pre-conceptional P-25OHD concentration on chances for pregnancy, as well as the effects of P-25OHD concentration during pregnancy on the risk of miscarriage and pregnancy outcomes in term of birth weight,

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Received 6 January 2011; revised 8 November 2011; accepted 9 November 2011; published online 29 February 2012

birth length, head circumference and Apgar score. Moreover, we studied whether pregnancy and breastfeeding patterns affect maternal P-25OHD concentrations.

SUBJECTS AND METHODS

In a population-based controlled cohort study, we included 153 healthy Caucasian women, aged 25–35 years, with immediate pregnancy plans and 75 age-matched women with no pregnancy plans for the next 21 months. In the present paper, we focus on the effects of vitamin D status in the group of women with immediate pregnancy plans. Data on women without pregnancy plans are, however, included in order to assess whether pregnancy and/or breastfeeding affects vitamin D status. The design of the study has previously been reported in detail.[29] In brief, women were recruited by direct mailing to 11 175 randomly selected women within the population of 21 317 women aged 25–35 years living in the community of Aarhus, Denmark. We obtained names and addresses from the Danish Civil Registration System. Power calculations were based on estimated changes in bone mineral density during pregnancy and breastfeeding, as previously detailed.[29] A total of 561 responded positively, among whom 333 were excluded for various predefined reasons as detailed in Figure 1. Women were included between October 2006 and January 2008. Women planning pregnancy were excluded if they did not achieve pregnancy within ~6 months after inclusion ($n = 53$), although two women achieving pregnancy at 7.3 and 8.7 months after inclusion remained in the study. The women who conceived ($n = 92$) attended our outpatient clinic on 7 occasions; that is, at baseline (before pregnancy), 3 times during pregnancy (pregnancy weeks 11 ± 2, 22 ± 1 and 35 ± 2) and 3 times after giving birth (that is, 15 ± 7, 129 ± 12 and 280 ± 15 days postpartum). The 75 women without pregnancy plans followed a similar schedule with clinical visits at time points parallel to the pregnancy group that is, investigations were performed at inclusion and at 3, 6, 9, 11, 15 and 21 (± 1 to 2) months after inclusion. Also, 22 women without pregnancy plans dropped out between visits 2 and 7 because of personal reasons, and 3 women conceived after the fourth follow-up visit and were excluded. Accordingly, 50 women completed the entire study. In the group of women without pregnancy plans, visit 1 was in most instances carried out during winter time, whereas the recruitment of the women planning pregnancy was carried out throughout the year. Samples collected from women in the pregnancy group were all analyzed, independently
of whether the woman terminated her participation in the study prematurely. Samples from women in the control group were only analyzed for participants who completed the entire study ($n = 50$).

The study was performed according to the Helsinki Declaration II. The study was notified to the Danish Data Protection Agency No. 2004-41-4737)

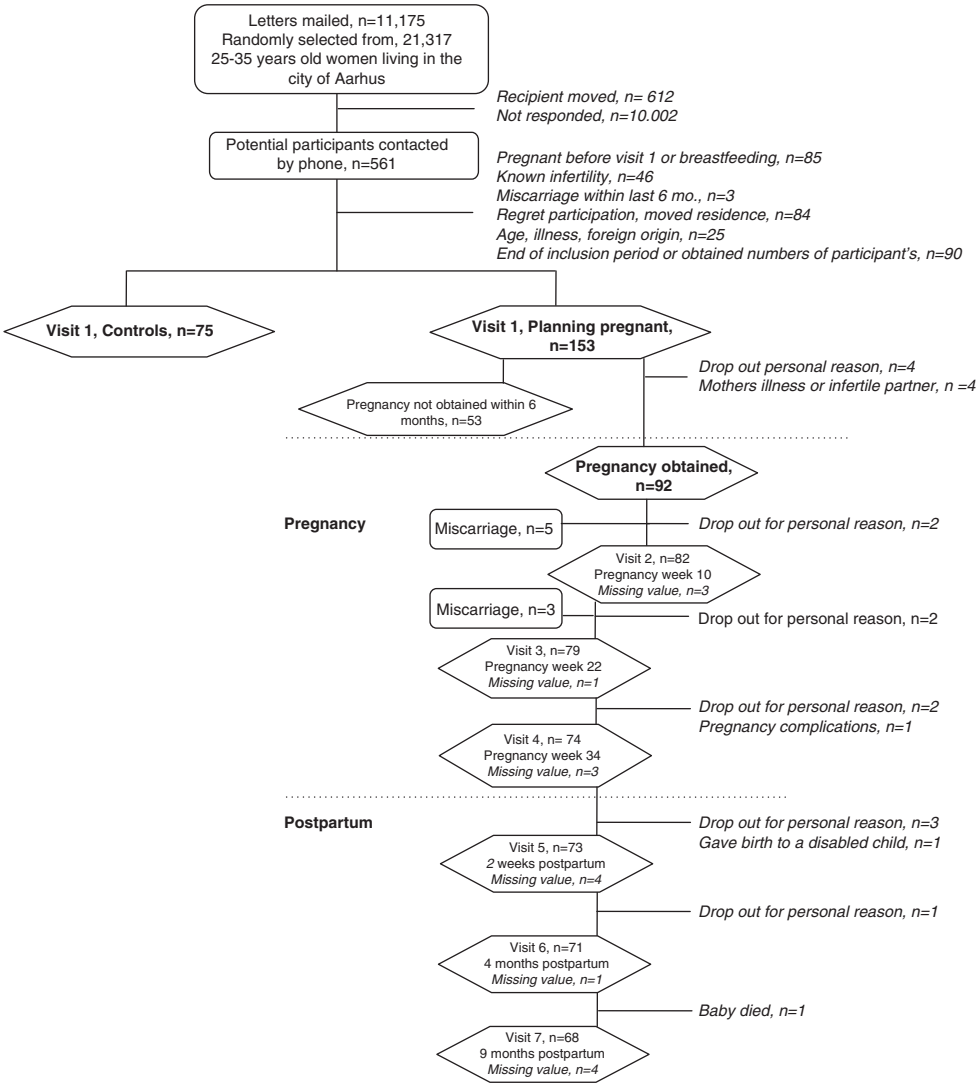

Figure 1. Study profile.

and approved by the Regional Scientific Ethical Committee of Aarhus County (No. 20040186).

Measurements

Body height and scale body weight were measured at inclusion and at each follow-up visit (Seca, Sa-med, Kvistgaard, Denmark) with women in light indoor clothing. Pre-pregnancy weight was used to calculate body mass index for each participant. A case report was drawn up for every participant, in which incident diseases and use of drugs were recorded. Participants were asked to fill in a non-validated internet-based questionnaire before each visit on diseases, use of drugs, smoking habits and use of calcium and vitamin D supplements, including multivitamin pills.

Confirmation of pregnancy

Pregnancy was confirmed by a pregnancy test carried out by the participant herself, followed by a self-booked appointment at the general practitioner, who confirmed pregnancy and referred the women to a routine ultrasound scan at the hospital at approximately pregnancy week 12.

Birth outcome

Gestational lengths and estimated date of delivery was calculated as 280 days after the first day of the last menstruation or according to the results of the ultrasound scan. All women gave birth at the Department of Obstetrics and Gynaecology, Aarhus University Hospital, Skejby, Denmark. At birth, data regarding the Apgar score, birth weight and length, head circumference and gestational length were obtained routinely by the midwives.

Biochemistry

At each visit, a non-fasting blood sample was drawn (between 0800 and 1400 h) with a minimum of stasis. It was centrifuged at 3000 r.p.m. for 10 min and thereafter stored at −80 °C within 1 h and analyzed blindly in the same run. Plasma 25OHD concentrations were analyzed by isotope dilution liquid chromatography-tandem mass spectrometry by a method adapted from Maunsell *et al*.[30] and described in detail.[31] The method quantifies $25OHD_2$ and $25OHD_3$, including the 3-epimer form that is not separated from $25OHD_3$. Calibrators traceable to NIST SRM 972 (ChromSystems, Gräfelfing, Germany) were used. Commutability was confirmed directly to NIST SRM 972 levels 1–4, and the sum of $25OHD_3$ and its epimer was compared. Mean coefficients of variation for $25OHD_3$ were 6.4% and 9.1% at levels of 66.5 and 21.1 nmol/l, respectively, and for $25OHD_2$ the coefficient of variation values were 8.8% and 9.4% at levels of 41.2 and 25.3 nmol/l, respectively.[31]

Statistics

We explored differences between groups using χ^2 tests for categorical variables and a two-sample *t*-test or Mann-Whitney *U*-test for continuous variables, as appropriate, after testing for normal distributions.

Linear regression analyses were used to study associations between variables. We used logistic regression analyses to calculate odds ratios with 95% confidence intervals. Repeated measure analysis of variance was used to assess whether vitamin D levels changed during pregnancy and breastfeeding. Descriptive statistics are reported as medians with the 25th and 75th percentile (p25, p75), unless otherwise stated.

In Denmark (latitude 56N), endogenous vitamin D synthesis is only possible during summer time, that is, from approximately May to October. The seasons of the year were divided into summer time (June-November) and winter time (December-May).

All statistical analyses were performed using the Statistical Package for Social Sciences, SPSS 17 (SPSS Inc., Chicago, IL, USA) for Windows.

RESULTS

The median (min-max) age of the 203 included women was 29 (25–35) years. At baseline, anthropometric, diet and lifestyle characteristics did not differ between women planning ($n = 153$) and not planning ($n = 50$) pregnancy, except for the use of vitamin supplement, which was more frequent in women planning pregnancy. Moreover, weight and body mass index were slightly higher in the group of women planning pregnancy. Only women without pregnancy plans used hormonal contraceptives ($n = 36$). The median (p25, p75) P-25OHD concentration at baseline was 59 nmol/l (46, 71). P-25OHD levels were <50 nmol/l in 48 (31%) women, whereas only 19 (12%) had concentrations >80 nmol/l. Figure 2 illustrates P-25OHD during the study period. At baseline, despite the fact that the women without pregnancy plans were included at winter time, they had significantly ($P<0.001$) higher P-25OHD concentrations (70 nmol/l (56, 92)) compared with women with pregnancy plans (59 nmol/l (46, 71)). This was attributable to a significantly higher P-25OHD concentration (78 nmol/l (57, 96)) in women using oral contraceptives compared with controls not using oral contraceptives ($n = 14$; 62 nmol/l (45, 71); $P = 0.03$). However, P-25OHD concentrations did not differ between women in the control group not using oral contraceptives and women planning pregnancy ($P = 0.98$), and this was not changed by adjustment for differences in the time of year of inclusion of studied subjects. At baseline, 58% of the women planning pregnancy used vitamin D supplements (Table 1). During pregnancy, this number increased to 75%, and 15 days postpartum supplements were used by 66%.

P-25OHD showed the well-known seasonal variations, with higher concentrations during summer time (May to October:

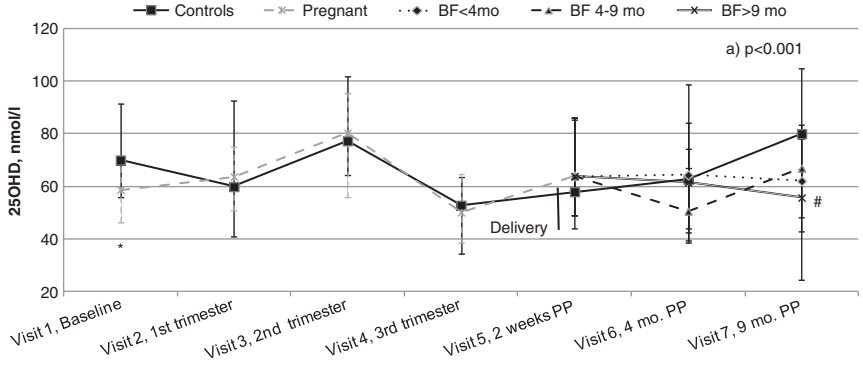

Figure 2. Changes in plasma levels of 25OHD during pregnancy and lactation. (a) Repeated measurement, difference between groups, all $P<0.001$. *Concentrations differ between women planning and not planning pregnancy ($P<0.001$). #Concentrations differ between women breastfeeding for longer than 9 months and the women not planning pregnancy ($P = 0.02$).

Table 1. Baseline characteristics of 153 Caucasian women planning pregnancy stratified by whether they achieved pregnancy

	All, n = *153*[a]	*Conceived,* n = *92*	*Failed to achieve pregnancy,* n = *53*	P-*value*
Plasma 25OHD (nmol/l)	59 (46, 71)	59 (48, 70)	57 (42, 74)	0.60
Age, years (min - max)	29 (25 - 35)	29 (25 - 35)	30 (25 - 35)	0.30
Parity, n *(%)*				
= 0	106 (69)	59 (64)	39 (74)	0.11
⩾1	47 (31)	33 (36)	14 (26)	
Height, cm (min - max)	167 (153 - 186)	167 (154 - 186)	168 (153 - 179)	0.99
Body weight, kg (min - max)	64 (48 - 114)	64 (48 - 114)	65 (49 - 109)	0.15
BMI, kg/m^2 (min - max)	23 (17 - 44)	23 (17 - 44)	24 (18 - 39)	0.08
Smokers, *n* (%)	30 (20)	15 (16)	11 (21)	0.30
Cigarettes per day, *n* (min - max)	6 (1 - 22)	7 (1 - 22)	6 (1 - 12)	0.54
Calcium intake, mg/day (min - max)	800 (350 - 2200)	750 (350 - 1900)	910 (350 - 2200)	0.06
Vitamin D supplementation				
Using supplementation, *n* (%)	89 (58)	49 (53)	35 (66)	0.20
Vitamin D dose, μg/day (min - max)	5 (1 - 30)	5 (1 - 30)	10 (2 - 15)	0.19

Abbreviations: BMI, body mass index; 25OHD, 25-hydroxyvitamin D. [a]Included eight women who dropped out (four dropped out for personal reasons and four due to illness or infertile partner); data on whether pregnancy was achieved or not are missing. Median with interquartile range (25th and 75th percentile (p25, p75)) or total range (min - max).

Table 2. Chances of achieving pregnancy according to plasma 25OHD concentration at baseline in terms of tertiles in 145 Caucasian women

Tertiles of P-25OHD	*P-25OHD (nmol/l) median (p25, p75)*	*Achieved pregnancy,* n *(%)*	*Crude OR (95% CI)*	*Adjusted OR (95% CI)*[a]
Lowest tertile, *n* = 48	40 (33, 46)	28 (56)	Reference	Reference
Mid tertile, *n* = 48	59 (54, 64)	34 (68)	1.7 (0.7, 4.1)	2.1 (0.8, 5.4)
Highest tertile, *n* = 49	76 (71, 89)	30 (59)	1.2 (0.5, 2.7)	1.4 (0.6, 3.4)

Abbreviations: CI, confidence interval; OR, odds ratio; p25, p75, 25th and 75th percentile; P-25OHD, plasma 25-hydroxyvitamin D. [a]Adjusted for mothers' age, baseline body weight, height, smoking status, season and parity (all dichotomized by medians and eliminated backward). Crude and adjusted ORs (OR (95% CI)).

65 nmol/l (54, 75), $n = 79$) than during winter time (November to April: 52 nmol/l (38, 64), $n = 74$; $P < 0.001$). The prevalence of P-25OHD concentrations <50 nmol/l was higher during winter time (45%) than during summer time (19%; $P = 0.001$), but only very few had severe P-25OHD deficiency in terms of P-25OHD concentrations <25 nmol/l ($n = 8$ at winter time).

P-25OHD concentrations and chances of pregnancy

A total of eight women dropped out because of personal reasons or illness or infertile partner before visit 2. In a few women ($n = 15$), the ultrasound scan revealed that the date of conception was before or just around the time of inclusion (median 12 (min to max: 0–36) days before inclusion). The success rate for becoming pregnant was 63% ($n = 92$), within median 1.3 (min to max: −1.2 to 8.7) months after baseline. Studied characteristics did not differ at baseline between women who conceived and those who did not achieve pregnancy (Table 1). In crude analyses, none of the studied indices predicted chances of pregnancy, including P-25OHD concentrations, parity, the age of the woman and the time spend from 'start trying to become pregnant' to the time of inclusion in the study. Neither did chances for conceiving differ between tertiles of P-25OHD levels. Results did not change after adjusting for age, body weight, height, smoking status, season, and parity (Table 2). However, in the adjusted analyses, the chance to achieve pregnancy was higher in multiparous compared with nulliparous women (odds ratio: 2.7; 95% confidence interval: 1.1–6.6%; $P = 0.03$) and in women <30 years compared with women >30 years of age (odds ratio: 3.1; 95% confidence interval: 1.4–6.9%; $P = 0.01$).

P-25OHD concentrations and pregnancy outcome

Among the 92 women who conceived, a miscarriage was encountered in 8 (9%) women. Five had a miscarriage before the visit at pregnancy week 10, and three had a miscarriage between the visit at weeks 10 and 22. Overall, P-25OHD concentrations at baseline did not differ between the eight women who had a miscarriage (54 nmol/l (38, 62)) and those who did not (62 nmol/l (49, 72); $P = 0.14$). However, women who had a miscarriage after pregnancy week 10 ($n = 3$) had lower P-25OHD concentrations at visit 2 (36 nmol/l (min–max: 35–54)) compared with those who did not have a miscarriage (65 nmol/l (24–111); $P = 0.03$). Studied characteristics did not differ at baseline between the women who encountered a miscarriage and those who successfully completed pregnancy.

During pregnancy, P-25OHD changed significant over time ($P < 0.001$), but similar changes occurred within the control group ($P < 0.001$), indicating no effect of pregnancy *per se* ($P = 0.59$; Figure 2). P-25OHD concentrations tended to increase in the second trimester followed by a decrease in the third trimester, but these changes did not attain statistical significance. Adjusting P-25OHD for the use of oral contraceptives within the control group or time of year of blood sampling at visit 2 (summer/winter season) did not change the results to any major degree.

The women ($n = 72$) completing visit 5 gave birth to children with a median (min–max) birth weight of 3595 g (2280–4900) with a birth length of 53 cm (46–56) and a head circumference of 35 cm(30–39) after 40.5 (35.6–42.0) weeks of pregnancy. One woman gave birth to a disabled child; no data on the size of the newborn were available. The majority of the babies (94%) had a 1-min Apgar score of >8 and all had a 5-min Apgar score of >8.

P-25OHD concentration measured at baseline, at any of the three individual follow-up visits, or the average P-25OHD concentration during pregnancy, did not affect gestational length or size of the newborn child in terms of birth weight, length or head circumference ($P > 0.09$). Neither did P-25OHD levels predict Apgar score, the number of small-for-gestational-age children (that is, $< 2.5\%$ of expected ($n = 32$)) nor the difference between expected weight for gestational age and actual birth weight ($P > 0.76$; data not shown).

Adjustments for age of the pregnant woman, height, weight changes during pregnancy, smoking status, parity, sex of infant and season of birth did not change the results ($P > 0.66$; data not shown). The time of year of gestation and time of year of giving birth (summer vs winter time) did not influence the studied indices ($P > 0.38$; data not shown). Finally, size of daily calcium intake did not influence the pregnancy outcome.

P-25OHD concentrations during breastfeeding

Postpartum, we categorized women *post hoc* according to breastfeeding status (yes/no), that is, whether women breastfeed for < 4 months ($n = 13$), 4–9 months ($n = 31$) or > 9 months ($n = 29$). Postpartum, P-25OHD levels changed significant over time within breastfeeding categories ($P = 0.04$), but 25OHD levels did not differ between breastfeeding categories ($P = 0.82$). Furthermore, similar changes occurred as a function of time within the group of non-pregnant women ($P < 0.001$). Accordantly, no differences between the controls and the women in the different breastfeeding categories were evident ($P = 0.11$; Figure 2). P-25OHD concentrations tended to decrease during prolonged breastfeeding with a slight increase after end of breastfeeding, but these changes were not statistically significant. Nevertheless, women breastfeeding for > 9 months had lower P-25OHD levels compared with the non-pregnant women at the last visit 9 months postpartum ($P = 0.02$), which was also the case after adjusting for season of blood sampling. Adjusting P-25OHD for the time of year of blood sampling at visit 5 (summer/winter season) did not change the results to any major degree. No difference in the unadjusted levels or season-adjusted levels were seen between any of the other groups at any of the three individual visits postpartum ($P > 0.30$).

DISCUSSION

In a group of healthy young Danish women planning pregnancy, severe P-25OHD deficiency (P-25OHD < 25 nmol/l) was rather uncommon, but most (88%) had P-25OHD < 80 nmol/l. To the best of our knowledge, the present study is the first investigation on possible associations between P-25OHD and human fertility. Our study showed no apparent association between chances of conceiving and vitamin D status as assessed by P-25OHD concentration. Nevertheless, similar to prior studies on predictors of fertility, we found chances of pregnancy to decrease with age and increase with parity.[32,33] Data from animal experimental studies have suggested a reduced fertility in female rats and mice with severe P-25OHD insufficiency.[1,2] However, this may rather be because of hypocalcemia than caused by low P-25OHD concentrations *per se*, as fertility seems to be restored when P-calcium concentrations were normalized by feeding the rats a diet rich in calcium.[1]

In several prior studies, low P-25OHD concentrations have been associated with an increased risk of adverse pregnancy outcomes, including an increased risk of pre-eclampsia and a low Apgar score at birth.[25,34,35] In a nested case–control study, P-25OHD concentrations in early pregnancy (< 22 weeks of gestation) were lower in women who developed pre-eclampsia compared with women who completed their pregnancy successfully.[34] Moreover, in a cross-sectional study[25] assessing daily vitamin D intake using a food-frequency questionnaire, the 1-min Apgar score was higher in newborns whose mothers had an adequate calcium and vitamin D intake compared with mothers with an inadequate intake.[25] In addition, a positive association has been reported between maternal P-25OHD concentrations at pregnancy weeks 28–32 and gestational length, independently of season.[36] In contrast, we found no effects of the levels of P-25OHD on gestational length or Apgar score. As none of our studied women developed pre-eclampsia, our data do not allow for conclusions on the risk of pre-eclampsia. However, the finding of lower P-25OHD concentrations at pregnancy week 10 in women, who later experienced a miscarriage, may support an effect of vitamin D status on the ability to complete a normal pregnancy. This is further supported by *in vitro* data showing that the vitamin D-activating enzyme (CYP27B1) and the vitamin D receptor are expressed in the placental syncytiotrophoblastic layer.[37] Moreover, calcitriol might affect the expression and secretion of human chorionic gonadotropin, progesterone and estradiol in cultured trophoblasts.[37] Accordingly, although our data do not allow for conclusions on cause–effect relationships, it seems biological plausible that low P-25OHD concentrations may exert adverse effects on the course of a pregnancy.[37,38]

Controversies exist on whether P-25OHD is affected by pregnancy *per se*. Holmes *et al.*[26] found lower P-25OHD concentrations in women during their second and third trimester compared with non-pregnant controls, whereas Hillman *et al.*[27] found no such pregnancy-induced decrease in P-25OHD. Neither do our data support an effect of pregnancy on P-25OHD concentrations, although our results are limited by the fact that most of our participants used vitamin D supplements.

Conflicting results have been reported on whether fetal development and growth is affected by vitamin D, as assessed by measuring dietary vitamin D intake,[19,25,39] effects of vitamin D supplementation[24,40,41] or P-25OHD concentrations.[7,17,23,24,36,40–42] Studies including low-income pregnant women[19] and women on a milk-restricted diet[39] have reported increased birth weight with increased vitamin D intake but, unfortunately, none of these studies reported P-25OHD concentrations. A positive association between P-25OHD concentrations and birth size has been reported in several studies, including mainly pregnant women with low P-25OHD concentrations,[7,24,40,42] whereas similar to our findings, a lack of association has been reported in studies including mostly P-25OHD-replete women.[7,16,17,23,25,36,41] Similarly, in a recent published randomized controlled trial, including women with a median P-25OHD concentration (mean ($\pm$s.d.)) between 58.2 and 61.6 nmol/l ($\pm$21.8 to 27.1), treatment with 10, 50, or 100 μg vitamin D3 per day from approximately pregnancy week 12 did not affect pregnancy outcomes compared with placebo.[43] Accordingly, it seems likely that an effect of P-25OHD concentration on pregnancy outcome, if present, is only clinically evident in case of vitamin D deficiency.

As the vitamin D content of breast milk depends on the maternal P-25OHD concentration, it may be assumed that lactation increases the need for vitamin D. Yet, similar to our overall results, in a study by Kent *et al.*,[28] P-25OHD concentrations did not change with lactation status. However, our findings of lower levels at 9 months postpartum in women lactating for > 9 months may suggest an increased loss of vitamin D, and thereby increased requirements during long-term lactation.

Strengths and limitations

The major strength of our study is the population-based design, recruiting women from the general population who planned pregnancy. By doing so, we were able to assess whether the current P-25OHD level in the general population of young Danish women affects their ability to become pregnant and risk of adverse pregnancy outcomes. Additionally, the design allowed for

multiple adjustments. The participants were randomly selected from the background population of 25- to 35-year-old women living in the community. Most likely, our cohort is representative of young Danish women as we found no major differences in studied indices between women planning or not planning pregnancy. However, we cannot exclude a 'healthy worker effect', that is, women who accept to participate in a clinical study like ours may differ in one or more aspects from nonparticipating women. Furthermore, women planning pregnancy may have a more conscious wish for a healthy lifestyle than women whose pregnancies are unplanned. The low prevalence of smoking and obesity, the relatively high daily calcium intake, frequent use of vitamin supplements, the relatively high P-25OHD levels, the few pregnancy-related complications and the long duration of breastfeeding compared with other studies[44,45] support the assumption that our studied women may represent a group of relatively healthy women compared with the average background population.

We started to include studied subjects in autumn 2006 and managed to include women not planning pregnancy at a much faster rate than women with pregnancy plans. Accordingly, women in the control group were included during winter time, whereas women with pregnancy plans were included throughout the year, thereby causing a potential bias due to seasonal variations in P-25OHD levels. Accordingly, we adjusted our analyses for time of year of blood sampling in order to avoid the effect of season on results.

Our findings of a similar pattern of variation in P-25OHD levels in pregnant/breastfeeding women and in the group of non-pregnant women indicates that vitamin D status is not affected to any major degree by pregnancy and lactation. Our findings emphasize the importance of including a control group, thereby avoiding misleading conclusions. Without a control group, we may easily have concluded (erroneously) that our findings in the group of pregnant/breastfeeding women were due to an effect of pregnancy/breastfeeding. Moreover, our finding is strengthened by the fact that 25OHD analyses were carried out as batch analyses, with samples from both groups analyzed concomitantly, thereby avoiding potential errors due to drift in the biochemical analyses.

As only few of our included women had severe vitamin D deficiency, our study does not allow for conclusions on effects of very low P-25OHD concentrations. Moreover, our study does not exclude minor effects of P-25OHD insufficiency that we may not have been able to detect because of the relative small size of our study.

An important limitation to our study is the lack of validated methods to estimate the dietary vitamin D intake and dermal vitamin D production. However, we do not expect the eligible women to differ markedly from the Danish background population with a mean dietary vitamin D intake of 2.5–4 μg/day. The best estimate of individual vitamin D status, that is, the combined effect of dietary intake, dietary supplementation and sun exposure, is considered to be plasma concentrations of 25OHD.[5,46] In the present study, blood samples were collected according to standardized procedures and analyzed blindly, minimizing the risk of information bias and pre-analytic variation.

Pregnancy was confirmed by a pregnancy test carried out by the participants themselves. Time of menstruation was not recorded and monthly blood sampling on human chorionic gonadotropin levels was not performed. Accordingly, potential early miscarriages may have been missed.

In conclusion, in a group of healthy young women without severe P-25OHD deficiency, recruited before a planned pregnancy, P-25OHD concentrations did not affect fertility or pregnancy outcomes. Neither did pregnancy or breastfeeding affect P-25OHD concentrations to any major degree. However, women with a miscarriage after pregnancy week 10 had relatively low P-25OHD concentrations at pregnancy week 10, which may indicate an effect of vitamin D on the ability to complete a course of pregnancy without complications.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We are grateful for the financial support provided to the project from The Danish Agency for Science, Technology and Innovation, The Aarhus University Research Foundation, AP Moeller Foundation for the Advancement of Medical Science, Svend Fældings Humanitære Fond, The Lundbeck Foundation, Aarhus University Fellowship and Helga and Peter Kornings Foundation.

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